DATA EVALUATION RECORD

INDAZIFLAM (AE 1170437; BCS-AA110717)

Study Type: §83-6, Developmental Neurotoxicity Study in Rats

Work Assignment No. 5-1-203 L (MRID 47443311)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 080818 **DP BARCODE:** D356856
TXR #: 0054980

TEST MATERIAL (PURITY): Indaziflam (93.14% a.i.)

SYNONYMS: AE 1170437; BCS-AA10717; *N*-[(1*R*,2*S*)-2,3-dihydro-2,6-dimethyl-1*H*-inden-1-yl]-6-(1-fluoroethyl)-1,3,5-triazine-2,4-diamine; Indaziflam

CITATION: Sheets, L.P. and R.G. Gilmore (2008). A developmental neurotoxicity study with technical grade BCS-AA10717 in Wistar rats. Bayer CropScience LP, Stilwell, KS. Laboratory Study/Report Nos.: 07-D72-KC/201877, July 1, 2008. MRID 47443311. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 47443311) Indaziflam (AE 1170437; BCS-AA10717; 93.14% a.i.; Batch No.: EFIM000511) was administered in the diet to pregnant Wistar Han rats (30/dose) from gestation day (GD) 6 through lactation day (LD) 21 at doses of 0, 150, 1000 or 7000 ppm. Beginning on LD 4, the high dose dietary level was reduced to 4000 ppm. The average test substance intake was 13.0, 83.8 or 432 mg/kg/day. Pups were not directly dosed. Dams were allowed to deliver naturally and were killed at weaning on LD 21. On PND 4, litters were standardized to 4 pups/sex/litter (when possible). The remaining offspring and dams were sacrificed and not examined further. One male and/or female per litter (approximately 16 – 20, minimum 10)/sex/dose, representing at least 20 litters/dose) were assigned to the following sets: motor activity (Set A), auditory startle (Set B), passive avoidance, water maze and functional observational battery (Set C). On PND 21, the whole brain was collected from a separate group of randomly selected offspring (Set D; 10/sex/dietary level; representing 20 litters/dose) for micropathologic examination and morphometric analysis. The remaining pups assigned to Set D (~10/sex/dose) were reserved for possible use as replacement animals or sacrificed on PND 21 without necropsy examination.

In the maternal animals: No treatment-related mortality occurred. Toxicity was only observed in the 7000/4000 ppm group. Treatment-related clinical signs were evident beginning on GD 6 as coarse tremor, dilated pupils, nasal stain and repetitive chewing movements in one animal. In the FOB, treatment-related effects were first evident on GD 6, and included dilated pupils,

dilated pupils that were unresponsive to penlight, tremor and repetitive chewing movements.

Body weights were decreased on GD 13 and 20 by 7-13%, resulting in a decreased cumulative body weight gain (GD 0-20) of 42%. Body weights were decreased throughout lactation by 4-14%, but increased cumulative body weight gain (LD 0-21) of 71% was noted. Body weight for females was reduced an average 14% and 11%, compared to controls, on LD 0 and 4, respectively. After LD 4 (when dose was reduced), the difference in body weight for the treated animals compared to controls progressively decreased with time. An effect on food consumption may have been observed during GD 6-13 but this was unclear due to food spillage. There was no effect on food consumption after reduction of the high-dose for the remainder of lactation. The number of litters was decreased by 17%. **The maternal LOAEL is 7000/4000 ppm (equivalent to 432 mg/kg/day), based on clinical signs and decreased body weights and body weight gains. The maternal NOAEL is 1000 ppm (equivalent to 83.8 mg/kg/day).**

In the offspring: No treatment-related effects were observed on litter size, viability, clinical signs, developmental landmarks, functional observational battery, auditory startle reflex, learning and memory testing, ophthalmology, nervous system morphometric evaluation, or gross or microscopic pathology

Decreased offspring body weights were noted in both sexes up to PND 4, post-culling (decr 14-17%), and up to PND 21 in both sexes (decr 5-10%). Offspring postweaning male body weights were decreased by 3-8%; treated group female body weights were similar to controls.

Motor activity was decreased in the 7000/4000 ppm males on PND 21 by 29%. Although there was no treatment-related effect on any measure of activity in high-dose males at other ages or in females at any age or dietary level, this difference from controls is considered treatment-related because it was statistically significant and the average results for the high-dose males (228) was below the range of historical control from four developmental neurotoxicity studies (261-341) conducted during the same time period (2005-2007), while the results for controls were well within this range. All other aspects of motor activity, including habituation, were not considered to be affected by treatment. Minor (3-5%) decreases in perfused and/or non-perfused brain weights in 7000/4000 ppm male and female pups were not considered adverse due to their small magnitude and change only at the limit dose: furthermore, most did not achieve statistical significance and in males, decreases were seen at PND 21 but not at termination.

The offspring LOAEL is 7000/4000 ppm (equivalent to 432 mg/kg/day), based on decreased body weights (males and females) and decreased motor activity in male pups on PND 21. The offspring NOAEL is 1000 ppm (equivalent to 83.8 mg/kg/day).

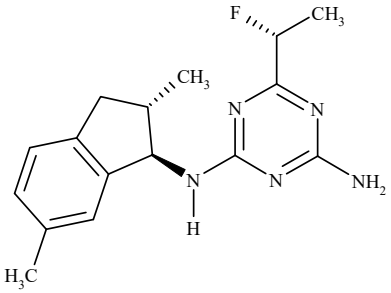
This study is classified as **Acceptable/Nonguideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 review of the positive control data.

COMPLIANCE: Signed and dated GLP Compliance, Quality Assurance, Flagging and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AE 1170437

Description:	Light beige powder
Batch No.:	EFIM000511
Purity (w/w):	93.14% a.i.
Stability of compound:	The Sponsor stated that the test compound was stable stored at room temperature and analyses showed it was stable in the dietary formulations for at least 7 days at room temperature.
CAS #:	950782-86-2 (changed from 730979-19-8 effective November, 2007)
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Wistar Han Crl:WI(Han)
Age and mean weight at study initiation:	11 weeks of age at cohabitation; 187-240 g females; weight of males not reported (not evaluated during study, only used for mating)
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing:	Suspended stainless steel cages with deotized cage board in the bedding tray; individually, except with one male each during co-habitation. Individually in plastic cages with corn cob bedding during gestation and lactation.
Diet:	Purina Mills Certified Rodent Diet 5002 in meal form, *ad libitum*, except during neurobehavioral testing.
Water:	Tap water, *ad libitum*, except during neurobehavioral testing.
Environmental conditions	
Temperature:	18-26ºC
Humidity:	30-70%
Air changes:	>10/hour
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	≥6 days

B. STUDY DESIGN

1. In life dates: Start (initiation of dosing): May 20, 2007
End (terminal sacrifice): August 24, 2007

2. **Study schedule:** The P generation females were mated and assigned to the study as they were determined to be sperm positive. The test substance was administered via the diet from gestation day (GD) 6 through lactation day (LD) 21, at which time the pups were weaned and the dams were sacrificed. On postnatal day (PND) 4, the litters were standardized by random selection of 4 males and 4 females per litter (as closely as possible). Pups that were not selected for the F1 generation were killed and discarded. Selected offspring were sacrificed on PND 21 for brain weight or neuropathological evaluations, and the remaining offspring were sacrificed at study termination on PND 75 (±5 days).

3. **Mating procedure:** The animals were time mated by co-housing one female with one male, for a maximum of five consecutive days. Each morning during the co-habitation phase, the dams and cages were examined for a vaginal plug, and vaginal smears were taken and examined for the presence of sperm. The day on which insemination was observed in the vaginal smear was designated GD 0 for that female. On Day 0 of presumed gestation, the female was removed and housed individually in a plastic nesting cage with corn cob bedding. Typically, females that were not sperm positive after the co-housing period or otherwise not placed on study were sacrificed without a necropsy examination.

4. **Animal assignment:** P generation females were weighed and those with body weights more or less than 20% of the mean weight were rejected. The remaining females were assigned to the control or exposure groups in sequence, as they were determined to be inseminated. P generation males served only as breeders. As such, they had no specific weight requirements and were arbitrarily selected for co-housing with females.

   Offspring were assigned to testing subgroups at the time of litter standardization on PND 4 (Table 1). An animal allocation program written in SAS was used to assign offspring to the following four sets (designated A-D) for assessment at each age. One male and/or female per litter (approximately 16 - 20 (minimum 10)/sex/dietary level, representing at least 20 litters per level) were assigned to the following sets: motor activity (Set A), auditory startle (Set B), passive avoidance, water maze and functional observational battery (Set C). On PND 21, the whole brain was collected from a separate group of randomly selected offspring (Set D; 10/sex/dietary level; representing 20 litters per level) for micropathologic examination and morphometric analysis. The remaining pups assigned to Set D (~10/sex/dietary level) were reserved for possible use as replacement animals or were otherwise sacrificed on PND 21 without necropsy examination.

   At approximately 50-60 days of age, randomly selected offspring animals (a minimum of 10/sex/dietary level, representing at least 20 litters per level) from Sets A, B and C were subjected to an ophthalmologic examination. At termination (PND 75±5 days), these animals were anesthetized and sacrificed by perfusion, with neural and muscle tissues collected for microscopic examination. Also at termination on PND 75±5 days, brains were collected from additional randomly selected animals (10/sex/dose group; representing 20 litters per level). These brains were weighed (fresh tissue weight) and discarded. The remaining animals assigned to sets A-C were sacrificed without routine gross necropsy examination or collection of tissues.

TABLE 1. Study design [a]

Experimental Parameter	Subset	Dose (ppm)			
		0	150	1000	7000/4000 [b]
Maternal Animals (P)					
No. of dams assigned	NA	30	30	30	30
Mean daily intake (mg/kg/day)	NA	0	13.0	83.8	432
FOB (GD 13 and 20)	NA	30	30	30	30
FOB (LD 11 and 21)	NA	10	10	10	10
Offspring (F_1) [c]					
Motor activity (PND 13, 17, 21, 60±2)	A	20/sex	20/sex	19-20/sex	19/sex
Auditory startle habituation (PND 23, 60±2)	B	19-20/sex	20/sex	20/sex	19/sex
Passive avoidance (PND 23 and 30)	C	16/sex	16/sex	16/sex	15-16/sex
Water maze (PND 60±2 and 7 days later)	C	16/sex	16/sex	16/sex	15-16/sex
FOB, detailed clinical exam (PND 4, 11, 21, 35±1, 45±1, 60±2)	C	20/sex	19-20/sex	20/sex	19/sex
Brain weight, perfusion, neuropathology and morphometric analysis (PND 21)	D	10/sex	10/sex	10/sex	10/sex
Ophthalmologic examination (~PND 50-60)	A, B, C	~10/sex	~10/sex	~10/sex	~10/sex
Perfusion and neuropathology (PND 75±5)	A, B, C	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination
Brain weight (PND 75±5) [d]	A, B, C	10/sex	10/sex	10/sex	10/sex

a Data obtained from pages 19 and 28 of the study report.
b The 7000 ppm dietary level was reduced to 4000 ppm on LD 4, due to excessive toxicity.
c Unless otherwise indicated, 1 male and/or female pup/litter was used (~16-20 [minimum of 10]/sex/dose, representing at least 20 litters).
d Remaining animals not used for neuropathology evaluations.
NA Not applicable

For FOB and motor activity testing, the same individual animals were evaluated at all scheduled time points. For the selection of animals and testing paradigms for cognitive (learning and memory) assessment, the same animals were used for assessments at the weanling and adult ages, but different tests were used at the two ages (see Observations section for details).

5. **Dose-selection rationale:** Doses were selected on the basis of results from a pilot reproductive toxicity testing study conducted with the test substance at doses of 0, 200, 1,000, 3,000 and 8,000 ppm (MRID 47443315, concurrently submitted and summarized in the DER for the rat reproduction study, MRID 47443293). In that study, declines in body weight for the 8,000 ppm females were evident throughout premating, gestation and lactation phases. These rats also had declines in food consumption during the premating period, with initial declines during the first week of treatment and continuing through week eight.

Declines in the 8000 ppm mean female terminal body weight and uterine weight were evident, as well as a trend toward increased thyroid weight. Adult male organ weight effects included increased absolute and relative liver weight in both the 3,000 and 8,000 ppm dose groups and increased absolute and relative adrenal and kidney weight in the 8,000 ppm dose group. In the 8,000 ppm dose group, female pup weight declines were evident on Day 21, with a decline in weight gain observed on Days 7-14 and Days 14-21. Organ weight declines were observed in the spleen and uterus of the female pups of this same dose group. Based on these combined results, the dietary levels selected for this developmental neurotoxicity study were 0, 150, 1000 and 7000 ppm. The 7000 ppm dietary level was selected as a maximum dose the animals would tolerate without excessive toxicity. The 1000 ppm dietary level was selected as an intermediate dose that might produce slight effects and the 150 ppm level was not expected to produce any treatment-related effects and therefore was selected to establish an overall NOAEL. Pharmacokinetics/metabolism (MRID 47443312), acute neurotoxicity (MRID 47443310) and subchronic neurotoxicity (MRID 47443309) studies were also concurrently submitted.

6. **Dosage administration:** The dose formulations were provided to the dams from GD 6-LD 21.

7. **Dosage preparation and analysis:** Formulations were prepared weekly by mixing appropriate amounts of the test substance in the diet and were stored at approximately -24ºC. Fresh dietary formulations were presented to the animals each week. A given batch of feed was available for *ad libitum* consumption for a period of one (GD 0 - LD 21) or two (post-weaning) weeks prior to changing. Dietary concentrations were not adjusted to correct for purity (percent active ingredient) in the test substance; however, concentrations were reduced during lactation to maintain a more constant level of exposure (mg/kg/day). After Day 21 of postnatal development, untreated feed was provided for consumption to all F1-generation animals that were retained on study.

   Concentrations of the test substance in the diet at each dose were measured by LC-MS/MS, using five batches of feed used in this study. The stability (at room temperature and freezer conditions) and homogeneity of the test substance in the feed were verified in a previous study [1] at dietary concentrations of 20 and 10,000 ppm, which bracketed those in the current study. The Sponsor stated that the dosage preparations were determined to be homogeneous and stable for 7 days at room temperature and 63 days at freezer conditions, but data were not presented in this study.

Results

Concentration (range as % of nominal): 95-109%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable, provided that the cited

[1] Jensen, T.L., "A Homogeneity and Stability Study of AE1170437 Technical in Rodent Ration," Bayer CropScience LP Report Number 201737, 2007.

stability study did indicate that the test compound was stable and homogeneous under conditions of the study.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals

1) Clinical observations: P generation males and females were observed (cage-side) for clinical signs at least once daily.

2) Detailed observations: A detailed evaluation of the dams for clinical signs with a physical examination was conducted once daily from the initiation of exposure (GD 6) through LD 21. These observations were performed by an individual who was aware of the animal's dosage group assignment.

3) Functional observational battery (FOB): Animals that were presumed to be pregnant (approximately 30 per dietary level) were subjected to a FOB on GD 13 and GD 20 and a minimum 10 dams/dietary level that were maintained on study with suitable litters were also observed on LD 11 and LD 21. All observations were performed by an individual who was unaware of each animal's dose group assignment. This evaluation was performed under standard animal room conditions (temperature, relative humidity, etc.) and included observations in the home cage, during handling and outside the home cage in an open field (one minute), using standardized procedures. Since it was not feasible for one person to evaluate all animals on all test occasions, the laboratory maintains evidence of inter-observer reliability for individuals who were involved with performing these observations.[2; 3] This observational battery included, but was not limited to, assessments (with severity scoring) of lacrimation, salivation, piloerection, exophthalmia, urination, defecation, pupillary function, palpebral closure, convulsions, tremor, abnormal movements, unusual behaviors and posture and gait abnormalities.

[2] Sheets, L.P., "Verification of Personnel Training to Perform a Functional Observational Battery with Rats," Bayer CropScience LP Report Number G200166, 2004.

[3] Sheets, L.P., "Historical Control and Method Validation Studies in Rats for the Acute and Subchronic Neurotoxicity Screening Battery", Bayer CropScience LP (formerly Miles Inc., Agriculture Division) Report 103979, MRID Number 427703-01, 1993.

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth and any other observations that may facilitate interpretation of the data.

4) **Body weight and food consumption:** Body weight and food consumption were measured once weekly during gestation and lactation, as follows: GD 6-13 and 13-20; LD 0-7, 7-14 and 14-21. In addition, dams were weighed on GD 0 and LD 4. Body weight gain was reported for GD 0-20. Measures of food consumption may have included consumption by the pups, especially during the third week of lactation.

5) **Delivery and culling:** Each dam was evaluated daily for evidence of delivery from GD 20 to the completion of delivery, which was designated LD 0 for the dam and PND 0 for the pups. Litter size (the number of pups delivered) and pup status (live or dead) at birth were recorded for each litter. If a dam delivered fewer than three pups per sex or if the litter size decreased to fewer than seven pups by PND 4, the dam and litter were sacrificed without necropsy examination. For litters that met the minimum size requirements, the size of each litter was adjusted on PND 4 to yield, as closely as possible, four males and four females. If there were more than 23 acceptable litters for any dietary level, the surplus litters were sacrificed on PND 4 after weighing without routine necropsy, with preference given to retaining litters with a full complement of four males and four females. Culled dams and pups were sacrificed by carbon dioxide (CO_2) asphyxiation and decapitation, respectively. Dams with insufficient litters were also sacrificed by CO_2 asphyxiation.

6) **Termination:** P generation males and females were sacrificed by CO_2 asphyxiation. A gross necropsy examination was not performed on P generation animals. Following co-habitation, males were sacrificed and discarded. Dams were sacrificed on LD 21, following the weaning of their respective litters. Females that were sperm positive and/or had an internal vaginal plug, but did not deliver, were generally sacrificed on GD 24 without necropsy examination.

b. Offspring

1) **Litter observations:** Live pups were counted and sexed on PND 0, 4, 11, 17 and 21 and once weekly thereafter. Daily throughout pre-weaning, offspring were examined cage-side for gross signs of mortality or morbidity. After PND 21, mortality was checked twice daily. More detailed observations for clinical signs were made once daily before weaning and once weekly thereafter. These observations were performed by an individual who was aware of assignments to dietary level.

2) **Developmental landmarks:** Male offspring were examined daily for balanopreputial separation, beginning on PND 38 and female offspring were examined daily for vaginal patency beginning on PND 29. Beginning on PND 4, selected males and females were tested daily for surface righting. On PND 21, all pups were tested for the presence of pupil constriction.

3) **Body weight and food consumption:** Live pups were weighed individually for each litter on PND 0, 4, 11, 17 and 21, and once weekly thereafter. The individual pups were also weighed when vaginal patency or balanopreputial separation were first evident. Group mean body weights and body weight gains were reported for males, females and combined sexes. Body weight gains were reported for PND 0-4, 4-11, 4-17, 4-21, 11-17, 11-21 and 17-21. Food consumption was not measured for the pups.

4) **Neurobehavioral evaluations:** The test room used for motor activity, auditory startle habituation and passive avoidance conditioning was a standard animal room that was set to be maintained on the same light-dark cycle as the room in which animals were housed, with tests conducted during the light phase. The water maze testing was performed in the room where animals were housed. The order of testing and assignment of animals to specific test devices was semi-random, such that groups were balanced across test times and devices and no animal was tested more than once in the same device. One planned exception was that animals were purposely tested in the same water maze on both occasions, as per standard procedure.

   i) **Functional observational battery (FOB):** On PND 4, 11, 21, 35 (±1 day), 45 (±1 day) and 60 (±2 days), approximately 20 offspring/sex/group (representing at least 20 litters per level; Subset C) were examined outside the home cage in an FOB assessment. This evaluation was performed according to the procedures described for maternal animals (see above). The only difference is that the neonates (i.e., PND 4 and 11) were not evaluated in the open field.

   ii) **Motor activity testing:** Motor activity was measured for approximately 20 rats/sex/dose (representing at least 20 litters per level; Subset A) on PND 13, 17, 21 and 60 (±2 days). The same offspring were evaluated in the figure-eight maze for 60 min (six 10 min intervals) at each time point, using a computer-automated system (Universal Maze Monitoring System, Version 1.41, Columbus Instruments, Columbus, OH) and personal computer for automated data collection. Each maze consisted of a series of inter-connected alleys (approximately 10 x 10 cm in cross-section) converging on a central arena and covered by transparent acrylic plastic. Each maze had eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys) to measure activity and an activity count was registered each time a beam was interrupted. The floor of each maze rested above absorbent paper, which was changed routinely at the end of each day. Broad-spectrum background noise [74±2dB(A)] was provided throughout the test to minimize acoustical variations during testing. The uniformity of light intensity (100±70 Lux) over each maze was verified daily. Motor and locomotor activities were examined as total activity counts (beam interruptions) for the 60-min session and as activity during each ten-min interval. Motor activity was measured as the number of beam interruptions that occurred during the test session.

Locomotor activity was measured by eliminating consecutive counts for a given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii) **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed in approximately 20 rats/sex/dose (representing at least 20 litters per level; Subset B) on PND 23 and 60 (±2 days). A personal computer was used to control the operation of an integrated startle response test system (Coulbourn Acoustic Startle, Version 3.210-00, Coulbourn Instruments, Allentown, PA) and for automated data collection. Groups of four animals (maximum) were tested simultaneously within each of two startle system enclosures. Each enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material, and houses a speaker mounted in a central position within the ceiling of the enclosure to provide the eliciting stimulus (S2) - a 50-msec burst (0 msec rise/fall) of broad-spectrum "white" noise [118±3 dB(lin)]. Each enclosure also houses four load cell/force transducer assemblies that are designed to measure the startle response. During the test session, animals were placed into individual restraining cages that were positioned on top of each load cell. The test session consisted of 50 trials that began following approximately a 5-min adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10-sec intervals. The peak response amplitude was determined for each trial as described below. The average response amplitude and the magnitude of decrease (habituation) over blocks of ten trials were compared among the dosage groups. Data collection began with the presentation of S2 and continued thereafter for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (i.e., one sample/msec for 200 msec) and converted to grams using a previously determined calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's individual response curve. Baseline was defined as the average force (g) exerted on the platform during the first 8 msec following the onset of S2, a time period that precedes response onset. This baseline value was taken to represent an approximate body weight measurement that was used to verify that the equipment used to measure the response amplitude was functioning properly. Response amplitude is defined as the maximum value of the average curve, minus the baseline (i.e., removing the animal's body weight from the measurement). Latency to peak is the time (msec) following the onset of S2 when the peak response amplitude occurs.

iv) **Learning and memory testing:** Learning and memory testing was performed in approximately 16 rats/sex/dose (minimum 10 offspring/sex/dose; Subset C). The same set of animals was used for testing passive avoidance (on PND 23 and 30) and water maze (PND 60±2 days and again seven days later).

Postweaning - passive avoidance: Animals were tested for acquisition on PND 23 and for retention on PND 30. Testing was conducted using equipment and computer programs from Coulbourn Instruments (Graphic State Notation 2 Version 2.002-00, Allentown, PA). Testing took place in individual isolation cubicles, each housing a single shuttle cage. Each isolation cubicle was lined with foam insulation to attenuate sound in the chamber and had a fan with a baffled air intake and exhaust system for

ventilation. The shuttle cage consisted of a Plexiglas and stainless-steel rectangular chamber fitted with front-loading access. Each shuttle cage (15 inches wide x 7.25 inches deep) was separated into two compartments of equal size (approximately 7 x 7 inches) by a wall that supported a centrally-located sliding (guillotine-type) door. The two compartments were identical, except that the walls in one compartment were lined with black film (dark-side) and the walls in the other compartment were not lined and it was illuminated during the test with a high-intensity lamp. The lamp was switched on to illuminate the light compartment at the start of each trial and remained on until either the animal crossed to the dark compartment or the trial ended. The floor of the cage consisted of a grid of stainless-steel bars. The movement of the animal from the starting (light) side to the dark compartment was detected by a photocell system. A Coulbourn solid-state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) distributed shock to the grid floor when the animal crossed to the dark compartment.

After adaptation, individual animals were placed individually into the "lighted" compartment of a conditioning apparatus (the shuttle cage), facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the non-illuminated side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered and the light switched off - signaling the end of that trial. At that time, the animal was returned promptly to the holding cage to wait for the next trial. If the rat failed to cross within 180 sec, it was returned to the holding cage and the latency assigned an arbitrary score of 180. This restriction dictated the use of nonparametric statistical analyses. The procedure was repeated until either the rat remained in the lighted compartment for 180 sec on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For this second trial, rats were placed in the illuminated side of the apparatus, given a 20-sec acclimation period and the latency to enter the dark side recorded. Animals that either failed to reach criterion performance within 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase of the experiment. The dependent measures were the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2 (learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Adult (PND 60) Offspring - Water maze: Animals were tested on PND 60 (±2 days), and again seven days later. Only animals that demonstrated acquisition were tested for retention. The water in the M-maze was maintained at 22 ±1°C. The mazes were constructed of opaque Plexiglas, with corridors approximately five inches wide and walls approximately 16 inches high with approximately 7.5 inches of water. This maze was selected as an established and widely-used device that can be used to measure associative learning and memory.

On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first (learning) trial, the rat was

required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (±5) seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Animals that satisfied the above criteria within the 15-trial limit were tested for retention seven days following acquisition (animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment). The correct goal and the criterion were the same for both sessions. Dosage groups were compared for the following dependent measures: Measures for acquisition included the number of trials-to-criterion, the average number of errors (incorrect turns in the maze) for each trial and the latency (in seconds) to reach the correct goal on trial 2 (a measure of short-term retention). Measures for retention included the number of trials-to-criterion, the average number of errors for each trial and the latency (in seconds) to reach the correct goal on trial 1 (a measure of long-term retention).

5) **Ophthalmology:** At approximately 50-60 days of age, ophthalmic exams were conducted using the males and females (a minimum of 10/sex/dietary level; representing at least 20 litters per level) that were selected for perfusion at study termination. If needed to clarify the significance of findings, the animals reserved for adult brain weight measurements were also subjected to ophthalmologic examination. The exam took place in a semi-darkened room. The pupillary reflex was tested using a penlight or transilluminator, with a mydriatic agent applied to each eye to dilate the pupil. The conjunctiva, cornea and lens were examined with a slit lamp microscope either before or after pupillary dilatation. After mydriasis, the vitreous humor, retina, choroid and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

6) **Postmortem observations**

   a. **Maternal animals:** Maternal animals were sacrificed by CO_2 asphyxiation on LD 21 following the weaning of their respective litters. The dams were discarded without postmortem examination. Females that were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed on GD 24 without necropsy examination.

b. Offspring

Necropsy: The offspring selected for brain weight or neuropathological evaluations were sacrificed on PND 21 (Subset D) or 75±5 days (Subset A, B and C). F1 generation animals that were found moribund (if any) while on study were sacrificed by CO_2 asphyxiation and underwent a gross necropsy examination. Tissues were collected at the discretion of the Study Director. In addition, randomly selected animals from Sets A-C that were used to measure fresh brain weight on PND 75±5 days were sacrificed by CO_2 asphyxiation and underwent a necropsy examination. Where required, the necropsy involved an examination of all organs (including the brain), body cavities, cut surfaces, external orifices and surfaces, with all gross abnormalities recorded. Gross lesions in neural tissues or skeletal muscle were appropriately sampled for microscopic examination. Other gross lesions were generally not collected for microscopic examination. Animals found dead (if any) underwent a necropsy examination and were disposed of without the routine collection of tissues.

Perfusion: Animals that were selected for perfusion on PND 21 (from Set D) or at study termination (from Sets A-C) were deeply anesthetized using an intraperitoneal dose of pentobarbital (approximately 50 mg/kg) and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by in situ fixation using universal fixative (1.0% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde) in phosphate buffer. On PND 21, only the brain (with olfactory bulbs) was collected. At study termination, the brain and spinal cord, eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle and both forelimbs were collected. All tissues were post-fixed in 10% buffered formalin. The brain was weighed upon removal from the skull, prior to placement into formalin, and the brain:body weight ratio calculated.

Measurements: Prior to sectioning the brain for histology, a Vernier caliper was used to obtain two linear measurements (mm).

1. Anterior-to-posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and
2. Anterior-to-posterior (AP) length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole.

These gross measurements were performed on 9-10 rats/sex/dose at PND 21 and 75±5 by an individual who was aware of dose group assignments.

Histology: Neuropathological examination was scheduled for rats in Set D (10 rats/sex/dose) at PND 21 and in sets A-C at PND 75±5. The brain tissue from perfused animals and any gross lesions collected at necropsy were further processed for microscopic examination. After the gross measurements were taken, the brain was divided into eight coronal sections for microscopic examination. The eight brain sections were processed according to standard procedures for paraffin embedding, sectioned at approximately 5 m and examined after staining with hematoxylin and eosin (H&E). In addition, the brain sections reserved for morphometric measurements (levels 3-5 and 7)

were stained using luxol fast blue/cresyl violet. Additional tissues were collected for microscopic examination from animals that were perfused at study termination. These included three levels of the spinal cord (cervical, thoracic and lumbar), the cauda equina, eyes, optic nerves and gastrocnemius muscle were embedded in paraffin and stained with H&E. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol methacrylate (GMA). GMA-embedded tissues were sectioned at 2 µm - 3 µm and stained using a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section. The CHECKED (X) tissues were evaluated for adult offspring.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES**
X	Forebrain	X	Sciatic
X	Center of cerebrum	X	Tibial
X	Midbrain	X	Sural
X	Cerebellum		**OTHER**
X	Pons	X	Lumbar dorsal root ganglion
X	Medulla oblongata	X	Lumbar dorsal root fibers
	SPINAL CORD	X	Lumbar ventral root fibers
X	Cervical swelling	X	Cervical dorsal root ganglion
X	Thoracic	X	Cervical dorsal root fibers
X	Lumbar swelling	X	Cervical ventral root fibers
	OTHER	X	Gastrocnemius muscle
X	Gasserian ganglion		
X	Cauda equine		
X	Optic nerve		
X	Eyes		

Micropathology and Morphometry: The tissues from high-dose animals were examined relative to those from the respective control group. If no treatment-related lesion was evident, further analysis was not performed. Any region where treatment-related neuropathology was observed underwent the following semi-quantitative analysis. Sections from all dose groups were coded and examined in randomized order without knowledge of the code. The frequency of each type of lesion was determined with the severity of each lesion graded.

Selected brain regions underwent the following quantitative analysis, with the individual performing the measurements aware of dose assignments. Initially, eight linear measurements were taken. If treatment-related effects were evident following this initial evaluation, then additional measurements may have been undertaken. Two of the seven measurements involved gross measurements of the intact brain, as described above. The other five were taken from the histologic sections using software calibrated with an ocular micrometer. These five measurements are described as follows:

1. Frontal cortex thickness (forebrain). This measurement was of the dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm.

2. Parietal cortex thickness (forebrain). This measurement was of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.

3. Caudate putamen horizontal width (forebrain; maximum cross-sectional width). This measurement was performed on the coronal section taken at the level of the optic chiasm.

4. Hippocampal gyrus thickness (midbrain). This measurement was of the full width of the hippocampal gyrus from the ventral tail of the dentate gyrus to the overlying subcortical white matter. Measurements were taken from the hippocampus from both sides of this section and the mean value was recorded.

5. Cerebellum height (cerebellum / pons). This measurement extended from the roof of the fourth ventricle to the dorsal surface.

In addition to these measurements, all brain sections from these control and high-dose male and female offspring underwent an extensive micropathologic evaluation. Morphometric data were collected on 9-10 rats/sex/dose at PND 21 and 75±5. Anterior-to-posterior cerebrum and cerebellum lengths were measured from rats in all dose groups. The frontal cortex, parietal cortex, caudate putamen, hippocampal gyrus and cerebellum were measured at the same time points in the control and 7000/4000 ppm groups.

D. DATA ANALYSIS

1. Statistical analyses: Group means were compared at the 5% and 1% levels of significance. Statistical analyses were performed using software from INSTEM Computer Systems, SAS and TASC. It was stated that continuous data were generally assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using an analysis of variance (ANOVA), followed by Dunnett's test when appropriate. In the event of unequal variances, Kruskal-Wallis test was used, followed by the Mann-Whitney U test when appropriate. Furthermore, additional statistical tests to assess continuous and frequency pathological data may have been used when deemed appropriate.

PARAMETER	STATISTICAL ANALYSES
Continuous FOB data	Data were analyzed by analysis of variance (ANOVA), with *post-hoc* comparisons using Dunnett's test.
Categorical FOB data	Data were analyzed using General Linear Modeling and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively.
Motor and locomotor activity	Session activity data for the four test occasions were first analyzed using an ANOVA to determine whether there was a significant day by treatment interaction. For days on which there was a significant treatment effect, Dunnett's test was used to determine whether the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine whether there was a significant treatment by interval interaction on each test occasion. For those test days, the data for each interval was subjected to analysis using Dunnett's test to determine whether the treated group was significantly different from the control.
Auditory startle response amplitude	Data (peak amplitude) for the two test occasions were first analyzed using an ANOVA procedure. If there was a significant group effect, Dunnett's test was used to determine whether the treated group was significantly different from control. The response amplitude data for each block of ten trials (five blocks/test session) were subjected to a Repeated-Measures ANOVA, using test block as the repeated measure. If there was a significant group by block interaction, the values for each block were subjected to analysis using Dunnett's test to determine if the results for treated animals were significantly different from control.
Passive avoidance	Latency data were analyzed using a Wilcoxon Test for time to failure (i.e., time to cross). The number of trials-to-criterion was analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning (acquisition) phase was analyzed as incidence data.
Water maze	Latency data were analyzed by a univariate ANOVA, with post-hoc analysis using Dunnett's test. The number of trials-to-criterion and the number of errors were analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning phase was analyzed as incidence data.
Organ weights Gross brain weights	Bartlett's test was performed on the data, followed by ANOVA (parametric) or Kruskal-Wallis (nonparametric), as appropriate.
Microscopic brain weights	ANOVA and/or t-tests were performed.
Ophthalmology Gross pathology	Data were first visually screened and if potential compound effects were suspected, then Chi-Square and one-tailed Fisher's Exact tests were used.
Micropathology	Chi-square Fisher's exact test was performed.

These statistical analyses were considered appropriate if parametric testing was used preferentially, but only when the assumptions of this type of testing were met. It is presumed that post-hoc analyses were also performed on the organ weights and gross brain weights. It is also presumed that the t-test performed on the microscopic brain weights used the pooled estimate of variance from ANOVA. Criteria for visual screening were not reported, and objective testing is preferred. A mixture of standard errors and standard deviations were reported; standard deviation is typically appropriate for pesticide toxicity tests.

2. Indices

a. Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating Index = No. of inseminated females/No. of females co-housed with males x 100

Fertility Index = No. of pregnant females/No. of inseminated females x 100

b. Offspring viability indices: The following viability (survival) indices were calculated from lactation records of litters in the study:

Live Birth Index = No. of live pups born per litter/Total no. of pups per litter x 100

Viability Index = No. of live pups on day 4 pre-culling per litter/No. of live pups born per litter x 100

Lactation Index = No. of live pups on Day 21 per litter / No. of live pups on day 4 post-culling per litter x 100

Positive and historical control data: Positive and historical control data (MRIDs 42770301, 46936202 and 46936203) submitted are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations:** No P generation females were found dead during gestation or lactation. Treatment-related clinical signs were evident in the 7000 ppm animals as coarse tremor in four females, beginning on GD 14, dilated pupils in seven females, beginning on GD 15 and nasal stain in three females, beginning on GD 6 (Table 2). There were no treatment-related effects at lower dietary levels. Treatment-related clinical signs during lactation were limited to dilated pupils in four high-dose females, which were apparent during gestation and resolved in all animals by LD 4 (when the dose was reduced to 4000 ppm) and repetitive chewing movements in one animal. Although chewing movements were apparent in only one animal as a clinical observation, it was evident by FOB in three 7000 ppm animals, which further supports this finding being a treatment-related effect. There were no treatment-related signs at lower dietary levels.

TABLE 2. Clinical signs [a]

Observations	Dose (ppm)			
	Control	150	1000	7000/4000 [b]
Gestation				
Coarse tremors	0	0	0	4
Dilated pupils	0	0	0	7
Nasal stain	0	0	0	3
Lactation				
Dilated pupil	0	0	0	4
Repetitive jaw chewing	0	0	0	1

a Data (n=30 up to GD 20; n=19-29 during lactation) were obtained from Table 2 on pages 67-69 and Table 5 on pages 74-76 of the study report.

b The 7000 ppm dietary level was reduced to 4000 ppm on LD 4, due to excessive toxicity.

Functional Observational Battery (FOB): Treatment-related findings were evident only in the 7000 ppm females. Treatment-related effects were first evident on GD 6, with dilated pupils in three females. On GD 20, the following observations were made (# affected/30): dilated pupils (6), dilated pupils that were unresponsive to penlight (2), tremor (1) and repetitive chewing movements (3). There were no treatment-related effects during lactation at any dietary level. Decreased numbers of urine pools were noted in the 7000/4000 ppm females at GD 6, GD 20 and LD 21; however, these minor decreases were not significant, were not considered adverse and may have been incidental.

TABLE 3. Functional observational battery in dams [a]

Observations/study day		Dose (ppm)			
		Control	150	1000	7000/4000 [b]
Gestation					
Dilated pupils	GD 6	0	0	0	3*
	GD20	0	0	0	6*
Slight repetitive chewing	GD 6	0	0	0	0
	GD 20	0	0	0	3*
Urine pools (mean±SD)	GD 6	1.7±1.7	1.6±1.6	1.3±1.4	0.9±1.1
	GD 20	1.4±1.2	1.3±1.1	1.4±1.1	0.9±1.0
	LD 21	1.4±2.1	1.0±1.5	0.6±0.8	0.7±1.6

a Data were obtained from Table 16 on pages 107-131 of the study report. n=30 on GD 6 and 20; n=10 on LD 21.
b The 7000 ppm dietary level was reduced to 4000 ppm on LD 4, due to excessive toxicity.
* Statistically different from control, $p \leq 0.05$

2. **Body weight and food consumption:** Summary body weight and food consumption data are reported in Table 4. Effects on body weight, body weight gain and food consumption were only observed in the 7000/4000 ppm group.

Gestation: Body weights were decreased ($p \leq 0.01$) on GD 13 and 20 (↓7-13%), resulting in a decreased ($p \leq 0.01$) cumulative body weight gain (GD 0-20) of 42%. During GD 6-13, food consumption increased ($p \leq 0.01$) by 159%. Although decreased body weight gain and increased food consumption suggests decreased food efficiency, the Sponsor stated that excessive feed spillage at the high dose occurred during the first week of treatment (GD 6-13) and resulted in an unreliable measure of food consumption for that week. Beginning on GD 13, grated feeders were given to all high-dose females to reduce feed spillage and thereby allow more accurate measurements of food consumption. Food consumption was similar to controls during GD 13-20. Therefore, the effect on food consumption is unclear.

Lactation: Body weights were decreased ($p \leq 0.05$, except on LD 14) throughout lactation by 4-14%, but increased (statistical analyses not performed [NP]) cumulative body weight gain (LD 0-21) of 71% was noted (calculated by the reviewer). Food consumption was decreased (NP) by 15% during LD 0-7, but was similar to controls thereafter. Beginning on LD 7, all 150 and 1000 ppm females were given grated feeders due to excessive feed spillage during the first week of lactation.

Body weight for high-dose females was reduced an average 14% and 11%, compared to controls, on LD 0 and 4, respectively. The Sponsor stated that based on substantively

reduced body weight (with associated concerns for the offspring) the high-dose was reduced on LD 4. After LD 4, the difference in body weight for high-dose animals progressively decreased with time. There was no effect on food consumption after the reduction of the high-dose for the remainder of lactation.

TABLE 4. Mean (±SE) maternal body weight and food consumption [a]

Observations/study day	Dose (ppm)			
	Control	150	1000	7000/4000 [b]
Gestation				
Mean body weight (g) GD 0	223.8±1.51	223.5±1.95	223.4±1.94	223.1±1.59
GD 6	238.2±2.25	241.2±2.18	237.9±2.87	238.1±2.64
GD 13	264.3±2.46	268.3±2.61	265.2±1.99	244.8±2.63** (↓7)
GD 20	324.6±4.12	329.8±3.52	325.0±2.90	281.4±4.06** (↓13)
Mean body weight gain (g) GD 0-20	100.8±3.30	106.4±2.45	101.5±1.92	58.3±3.65** (↓42)
Mean food consumption (g/animal/day) GD 6-13	19.2±0.54	19.1±0.42	18.8±0.41	49.7±3.37** (↑159)
GD 13-20	19.8±0.38	19.7±0.28	20.9±0.46	20.6±1.13
Lactation				
Mean body weight (g) LD 0	251.3±2.68	256.5±3.21	254.3±2.40	217.2±2.73** (↓14)
LD 4	267.9±4.59	269.8±2.48	269.7±2.66	238.0±3.52** (↓11)
LD 7	277.3±3.23	277.4±2.55	277.0±3.30	253.1±2.94** (↓9)
LD 14	289.2±4.97	293.2±2.48	293.3±3.03	277.3±3.17
LD 21	284.4±2.38	286.7±2.74	282.9±2.49	273.9±3.48* (↓4)
Mean body weight gain (g) LD 0-21 [c]	33.1	30.2	28.6 (↓14)	56.7 (↑71)
Mean food consumption (g/animal/day) LD 0-7	41.8±2.49	41.7±4.58	41.5±3.74	35.7±1.29 [d] (↓15)
LD 7-14	51.5±1.16	51.7±0.57	50.4±0.71	51.0±0.89
LD 14-21	61.9±0.84	62.6±0.72	60.9±1.08	62.0±1.07

a Data (n=18-30) were obtained from Tables 3-4 on pages 70-73 and Tables 6-7 on pages 77-80 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.
b The 7000 ppm dietary level was reduced to 4000 ppm on LD 4, due to excessive toxicity.
c Body weight gain during lactation was calculated by reviewers.
d The Sponsor stated that values were derived and were not subjected to statistical analysis.

3. **Test substance intake:** Based on maternal food consumptions, body weights and nominal dietary concentrations, the doses expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods are presented below (Table 5).

For high-dose females, spillage precluded a determination of test substance intake during the first week of exposure. However, the 568 mg/kg bw/day value for GD 13-20 is considered to be a representative measure of test substance intake for both weeks of exposure during gestation. For high-dose animals, the average daily intake for LD 0-4 and LD 4-7 was 559.4 and 437.9 mg/kg bw/day, respectively, as the dietary concentration was reduced. Relatively higher food consumption was noted with the reduction in dietary concentration. Taking the average of these two values for LD 0-7 along with the two values for LD 7-14 and LD 14-21, the average daily intake for high-dose animals was 432 mg/kg/day.

TABLE 5. Mean (±SE) maternal test substance intake (mg/kg body weight/day) [a]			
Period	Dose (ppm)		
	150	1000	7000/4000
Gestation			
Gestation days 6-13	12.3±0.23	81.6±2.42	1414.7±95.01 [b]
Gestation days 13-20	11.5±0.14	80.8±1.59	567.9±30.73
Lactation			
Lactation days 0-7	14.6±1.59	89.6±8.08	559.4/437.9 [c]
Lactation days 7-14	12.9±0.16	85.2±0.99	383.5±5.95
Lactation days 14-21	13.5±0.22	82.0±1.18	345.4±3.73
Average			
Average daily intake during gestation and lactation	13.0	83.8	432

a Data were obtained from pages 38-39 and Table 8 on pages 82-83 in the study report. Dietary concentrations were reduced during Weeks 1-3 of lactation (by factors of 1.7, 2.2 and 2.6, respectively), based on estimated increases in feed consumption (g consumed/kg body wt./day) during lactation.
b Unreliable measurement due to feed spillage.
c The highest dietary level was reduced from nominal 7000 ppm to 4000 ppm, beginning on LD 4. Values for high-dose animals for LD 0-7 were derived from calculations performed using MS Excel. Nominal concentrations were calculated using the complete isomeric total (purity 94.5%)

4. **Reproductive performance:** No intercurrent deaths were observed and the mean gestation duration in the treated groups was similar to control. No occurrence of dystocia was reported. Although there were 28-29 pregnant females per dose group that delivered litters, the number of litters actually evaluated was lower (19-23) due to culling based on the criteria outlined in "Observations," Section C.5.

TABLE 6. Reproductive performance [a]				
Observation	Dose (ppm)			
	Control	150	1000	7000/4000
Number mated	30	30	30	30
Number pregnant/litters born	28	28	28	29
Number of litters selected for evaluation	23	23	21	19
Intercurrent deaths	0	0	0	0
Mean (±SE) gestation duration (days)	21.9±0.09	21.7±0.12	21.9±0.12	21.9±0.07
Incidence of dystocia	0	0	0	0

a Data were obtained from Table 1 on pages 65-66 and Appendix 1 on pages 228-235 in the study report.

5. **Maternal postmortem results:** No postmortem findings were presented.

B. OFFSPRING

1. **Viability and clinical signs:** No treatment-related effects were observed on offspring litter size or viability (Table 7). No treatment-related clinical signs were observed cage-side.

TABLE 7. Litter size and viability [a]

Observation	Dose (ppm)			
	Control	150	1000	7000/4000
Total number born	252	249	220	205
Number born live	249	249	220	205
Number born dead	3	0	0	0
Sex ratio Day 0 (% males) [b]	53	50	56	50
Mean litter size:				
Day 0	11	11	10	11
Day 4 [c]	11	11	10	11
Day 4 [d]	8	8	8	8
Day 21	8	8	8	8
Live birth index [e]	98.8±0.64	100.0±0.00	100.0±0.00	100.0±0.00
Viability index [e]	99.6±0.43	97.2±1.69	99.6±0.43	99.1±0.63
Lactation index [e]	100.0±0.00	100.0±0.00	99.4±0.60	100.0±0.00

a Data obtained from Table 9 on pages 84-86 and Appendix 1 on pages 232-235 in the study report.
b Calculated by reviewers.
c Before standardization (culling).
d After standardization
e Mean (±SE)

2. <u>Body weight</u>: Decreases ($p \leq 0.05$) in offspring body weights were noted in both sexes at 7000/4000 ppm up to PND 4, post-culling (↓14-17%), and decreases (NS, except as noted) were noted up to PND 21 in the males (↓6-10%; $p \leq 0.05$ at PND 11 and 21) and females (↓5-9%; Table 8).

TABLE 8. Mean (±SE) pre-weaning pup body weights (g) [a]

PND	Dose (ppm)							
	Control	150	1000	7000/4000	Control	150	1000	7000/4000
	Males				Females			
1	6.2±0.09	6.0±0.10	6.2±0.11	5.3±0.09** (↓15)	5.8±0.09	5.6±0.10	5.9±0.11	5.0±0.08** (↓14)
4 [b]	10.3±0.30	10.2±0.32	10.3±0.20	8.6±0.26** (↓17)	9.7±0.28	9.7±0.36	10.0±0.23	8.2±0.25** (↓15)
4 [c]	10.3±0.30	10.2±0.33	10.3±0.20	8.5±0.26** (↓17)	9.7±0.3	9.6±0.36	10.0±0.23	8.2±0.24** (↓15)
11	25.7±0.58	25.8±0.66	25.4±0.44	23.1±0.60* (↓10)	24.5±0.64	24.9±0.69	24.9±0.47	22.4±0.61 (↓9)
17	39.6±0.78	40.1±0.73	39.2±0.63	37.1±0.65 (↓6)	37.9±0.82	38.4±0.67	37.7±0.56	35.7±0.68 (↓6)
21	50.6±0.94	50.4±0.89	50.0±0.91	47.0±0.74* (↓7)	48.1±1.06	48.6±0.87	48.3±0.73	45.8±0.82 (↓5)

a Data (n=19-23) were obtained from Table 12 on pages 93-96 in the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.
b Before standardization (culling).
c After standardization (culling).
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

Offspring postweaning male body weights were decreased by 3-8% ($p \leq 0.05$; except at PND 70; Table 9) at 7000/4000 ppm, while female body weights in the treated groups were similar to controls.

TABLE 9. Mean (±SD) post-weaning pup body weights (g) [a]

PND	Dose (ppm)							
	Control	150	1000	7000/4000	Control	150	1000	7000/4000
	Males				Females			
28	83.0±7.3	83.6±7.0	82.8±5.2	77.5±5.6* (↓7)	78.6±8.3	80.4±7.5	79.9±6.0	75.9±5.5
35	132.5±10.2	132.0±9.0	131.4±7.7	122.1±8.1* (↓8)	115.8±9.7	117.2±8.5	118.5±6.5	112.0±6.1
42	178.4±11.4	179.4±9.4	178.7±9.8	168.2±8.9* (↓6)	138.9±11.9	142.2±8.4	143.9±7.0	136.2±7.1
49	223.8±13.7	225.4±11.5	224.6±12.2	211.7±10.0* (↓5)	158.3±9.9	159.7±8.5	162.0±8.1	154.8±7.3
56	267.9±16.2	270.9±12.6	271.3±14.5	256.0±11.0* (↓4)	174.9±10.3	174.2±9.1	176.4±9.0	170.1±9.2
63	299.5±17.7	302.8±14.2	304.9±15.6	287.8±11.5* (↓4)	187.5±9.5	187.6±9.0	189.9±10.3	182.6±8.7
70	327.8±20.5	334.1±16.2	336.6±17.6	317.3±12.0 (↓3)	198.8±9.6	198.8±8.7	200.3±10.1	193.5±9.4

a Data (n=19-23) were obtained from Table 15 on pages 105-106 in the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.
* Statistically different from control, $p \leq 0.05$

3. Developmental landmarks

a. Sexual maturation: No treatment-related effects were noted on sexual maturation (Table 10a). Times to preputial separation and vaginal opening were similar between the treated groups and controls.

TABLE 10a. Mean (±SE) age of sexual maturation (days) [a]

Parameter	Dose (ppm)			
	Control	150	1000	7000/4000
Number rats/sex	23	23	21	19
Preputial separation (males)	42.2±0.43	43.2±0.46	42.7±0.43	43.3±0.38
Vaginal opening (females)	32.2±0.51	32.7±0.54	32.3±0.33	32.9±0.51

a Data were obtained from page 103 in the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.

b. Other developmental landmarks: No treatment-related effects were noted on time to pupil constriction or surface righting (Table 10b).

TABLE 10b. Mean (±SE) age at developmental landmark (days) [a]				
Parameter	Dose (ppm)			
	Control	150	1000	7000/4000
Number rats/sex	23	23	21	19
Pupil constriction	21.0±0.0	21.0±0.0	21.0±0.0	21.0±0.0
Surface righting	5.9±0.20	5.9±0.21	6.2±0.20	5.7±0.21 [b]

a Data were obtained from Table 14 on pages 102-103 in the study report.
b n=18

4. Behavioral assessments

a. Functional observational battery: No treatment-related effects were observed during the functional observational battery. The number of urine pools counted from males in the open field decreased (NS) with increasing dose at PND 21 and PND 35 and was also decreased at PND 45 at 7000/4000 ppm (Table 11). The numbers of urine pools in the treated groups were similar to controls at PND 60 in males and at all time points in females. This effect was minor, was not considered adverse and may have been incidental. The number of fecal boluses were decreased (NS) in the 7000 ppm males at PND 21 (0.5 treated vs 1.2 controls). Although this effect seemed dose-dependent, a similar effect was not observed later in the males nor at any time in the females.

TABLE 11. Male urination in the open field functional observational battery (mean # of pools ±SD) [a]				
Day observed	Dose (ppm)			
	Control	150	1000	7000/4000
-PND 21	1.2±1.5	1.0±1.3	0.7±1.5	0.6±1.0
-PND 35	1.0±1.3	1.0±1.1	0.7±1.2	0.6±1.0
-PND 45	0.8±1.4	0.7±1.1	0.8±1.4	0.4±0.8
-PND 60	1.6±1.3	1.2±1.0	1.5±1.5	1.6±1.7

a Data (n=19-20) were obtained from Table 17 on pages 142, 148, 154 and 160 in the study report.

b. Motor activity: Total motor and locomotor activities are summarized below in Tables 12-14.

The only treatment-related effect was a decrease ($p \leq 0.05$) in motor activity in the 7000/4000 ppm males on PND 21 (↓29%). Although this difference is relatively small and there was no treatment-related effect on activity in high-dose males at other ages or in females at any age or dietary level, it is considered treatment-related because the decrease was statistically significant and the average results for the high-dose males (228) was below the range of historical control from four developmental neurotoxicity studies (261-341) conducted during the same time period (2005-2007), while the results for controls (and other dose groups) were well within this range. Motor and locomotor activities increased with age, particularly from PND 13 to PND 17, and no decrease in activity with age was observed up to PND 60. Habituation was unaffected by treatment. Subsession motor activity for the males is included in the Attachment.
Differences from control that are considered to be unrelated to treatment occurred on

PND 17, with statistical and non-statistical decreases in motor activity at all dietary levels for males (↓34-39%) and females (↓21-32%), respectively. These differences in total activity counts per session are attributed to high levels of motor activity for control males (284) and females (276), relative to the treated animals and historical controls. By comparison, motor activity for controls at PND 17 in the aforementioned DNT studies ranged from 126-222 for males and 143-207 for females, which compares with values for treated males (174-188) and females (187-218) that were comparable or slightly above the range of these historical controls. Locomotor activity was similarly lower than control on PND 17 in males (33-41%) and females (20-32%) at all levels, without statistical significance. Combining this information for controls and treated animals with the lack of a dose-response over this large range of dietary levels supports that these differences are incidental and unrelated to treatment.

Motor and locomotor activity data were also subjected to analysis at each 10-min interval of the 60-min test session. Evaluation of the progressive decrease in activity over the course of a test session provides a measure of habituation. For motor activity, habituation was evident in control males and females at all ages. For locomotor activity, habituation was apparent in controls at all ages except PND 13, when locomotor activity was quite low for all six intervals of the test session.

An analysis of the data by test interval provided no further evidence of a treatment-related effect, relative to the results for the entire session. The levels of motor and locomotor activity were generally comparable to control for all test intervals on all test occasions. One minor exception occurred on PND 17 where activity levels were significantly decreased during interval 3 for motor activity in 1000 ppm females. This difference from control was considered to be incidental and unrelated to treatment because it was not related to dose and occurred during only one interval. The differences discussed above (for the entire session) involving the PND 17 (all levels) and PND 21 (high-dose) males were evident here as slightly lower levels of activity throughout the session, with the magnitude of habituation comparable to that of control.

TABLE 12. Mean (±S.D.) motor activity data (total activity counts for session) [a]

Test Day	Dose (ppm)			
	Control	150	1000	7000/4000
Males				
PND 13	69±44	85±84	99±63	117±93
PND 17	284±152	174±118* (↓39)	188±91* (↓34)	185±95* (↓35)
PND 21	322±100	272±124	259±88	228±71* (↓29)
PND 60	569±192	562±162	544±134	616±116
Females				
PND 13	60±73	65±58	75±60	81±65
PND 17	276±161	197±136	187±78	218±146
PND 21	292±88	306±101	294±110	279±114
PND 60	691±158	784±141	753±203	755±221

a Data (n=19-20) were obtained from Table 18 on pages 189-191 in the study report.

* Statistically different from control, p≤0.05

TABLE 13. Mean (±S.D.) locomotor activity data (total activity counts for session) [a]

Test Day	Dose (ppm)			
	Control	150	1000	7000/4000
Males				
PND 13	7±8	10±17	13±17	9±10
PND 17	76±44	45±32	51±30	50±29
PND 21	83±28	76±36	72±27	67±25
PND 60	395±151	391±133	372±105	426±100
Females				
PND 13	8±17	9±9	8±8	11±13
PND 17	75±54	60±47	51±24	57±36
PND 21	84±37	89±41	91±49	85±46
PND 60	489±128	523±117	470±131	494±152

a Data (n=19-20) were obtained from Table 19 on pages 192-194 in the study report.

TABLE 14. Historical controls for motor and/or locomotor activity data (total activity counts for session, mean±S.D.) [a]

Study No.	PND 17 – motor activity	PND 17 – locomotor activity	PND 21 – motor activity
Males			
05-D72-YF	179±110	38±28	341±80
06-D72-EV	222±103	55±25	315±134
06-D72-DH	156±123	32±30	331±150
07-D72-IL	126±71	32±22	261±80
Females			
05-D72-YF	162±157	37±38	NA
06-D72-EV	182±126	41±33	
06-D72-DH	143±82	36±29	
07-D72-IL	207±112	55±37	

a Data were obtained from page 49 in the study report.
NA Data not available

c. <u>Auditory startle reflex</u>: No treatment-related effect was observed on the auditory startle reflex (Tables 15a and 15b). Peak amplitude and latency in the treated groups were comparable to the controls. Habituation was demonstrated in all groups.

TABLE 15a. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (ms) in F1 male rats[a]

Dose (ppm)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5	Mean
PND 23							
Control	Peak Amp.	38±20	36±13	39±17	32±14	32±15	35±14
	Latency	39±5	38±6	37±5	38±6	37±5	37±5
150	Peak Amp.	37±15	36±17	36±15	31±16	27±13	33±14
	Latency	38±3	36±3	37±6	38±6	38±5	37±3
1000	Peak Amp.	46±17	44±20	41±18	39±14	35±16	41±16

TABLE 15a. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (ms) in F1 male rats[a]

Dose (ppm)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5	Mean
	Latency	38±5	37±5	37±5	37±7	37±4	37±4
7000/4000	Peak Amp.	37±15	37±15	39±12	34±11	32±10	36±12
	Latency	39±7	38±7	36±5	38±6	38±5	38±5
PND 60							
Control	Peak Amp.	272±149	282±167	248±148	193±145	148±97	229±132
	Latency	42±3	40±2	38±3	38±3	39±4	40±2
150	Peak Amp.	215±116	240±143	206±111	162±108	130±81	191±103
	Latency	43±5	43±6	39±3	41±5	40±5	41±4
1000	Peak Amp.	303±167	307±161	279±140	234±110	168±98	258±121
	Latency	40±3	39±2	38±2	38±2	38±2	39±2
7000/4000	Peak Amp.	246±133	249±130	198±114	156±86	106±76	191±98
	Latency	41±3	40±3	40±3	40±4	40±4	40±2

a Data (n=19-20) were obtained from Tables 22 and 23 on pages 213-220 in the study report.

TABLE 15b. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (ms) in F1 female rats[a]

Dose (ppm)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5	Mean
PND 23							
Control	Peak Amp.	43±19	44±20	41±20	39±21	36±14	41±18
	Latency	37±4	34±2	34±2	34±1	34±2	35±1
150	Peak Amp.	36±16	36±16	34±11	32±13	30±12	34±12
	Latency	39±6	37±8	35±4	36±4	37±4	37±4
1000	Peak Amp.	44±21	42±17	43±16	38±16	38±15	41±16
	Latency	36±3	35±5	34±1	34±3	34±2	35±2
7000/4000	Peak Amp.	38±17	38±16	38±17	36±17	32±15	36±15
	Latency	38±6	38±8	36±5	37±6	35±4	37±5
PND 60							
Control	Peak Amp.	136±82	147±109	106±73	89±68	67±50	109±70
	Latency	41±4	39±4	39±5	39±5	39±6	39±4
150	Peak Amp.	103±50	112±60	84±55	67±40	65±31	86±39
	Latency	43±3	42±3	42±6	42±5	41±5	42±3
1000	Peak Amp.	142±84	171±105	159±113	138±108	102±75	142±89
	Latency	42±4	40±3	39±3	37±4	39±5	39±3
7000/4000	Peak Amp.	111±85	118±92	99±80	92±75	72±56	98±70
	Latency	42±4	41±4	40±5	40±5	40±5	41±3

a Data (n=19-20) were obtained from Tables 22 and 23 on pages 213-220 in the study report.

d. Learning and memory testing

1. **Post-weaning passive avoidance:** No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance test (Table 16a). Acquisition and retention were clearly evident in control males and females. On the first test occasion, acquisition was evident in males and females as a marked increase in the latency to cross for the second trial (an average

174.0 and 180.0 sec, respectively), compared to the first trial (an average 58.6 and 35.3 sec, respectively). Thus, acquisition of the avoidance response (a failure to cross within the 180-sec time limit for a trial) was quickly attained in control females and males. As *per* standard procedure, animals that either failed to satisfy the criteria used to establish acquisition or to cross during the first two trials (and therefore never received the conditioning stimulus) were not tested for retention. There was one control male and no females that did not cross during the learning phase. On the second test occasion, which occurred one week after the first, retention was evident in control males and females as a protracted delay to cross within the 180-sec time limit of the first trial (an average 174.0 and 180.0 sec, respectively), compared to the first trial on the first test day (an average 58.6 and 35.3 sec, respectively). Retention was also evident in control males and females by a reduced average number of trials-to-criterion on the second test occasion (2.1 and 2.3 trials, respectively), compared to the first (3.2 and 3.0 trials, respectively). These comparisons (within the control group) to verify changes in performance with experience (acquisition/learning and retention/memory) were not subjected to statistical analysis.

TABLE 16a. Passive avoidance performance at PND 23 and 30 in offspring (mean±S.D.) [a]

Session/Parameter		Dose (ppm)			
		Control	150	1000	7000/4000
Males					
Session 1 (learning phase)	Trials to criterion	3.2±0.7	3.1±0.6	3.1±0.5	3.1±0.3
	Latency trial 1 (sec)	58.6±58.7	58.9±57.2	20.4±11.3	45.7±41.6
	Latency trial 2 (sec)	174.0±16.9	180.0±0.0	180.0±0.0	177.2±7.7
	Failed to meet criterion	0	0	0	0
	Failed to cross	1	1	0	0
Session 2 (retention phase)	Trials to criterion	2.1±0.4	2.2±0.6	2.1±0.5	2.3±0.6
	Latency trial 1 (sec)	174.0±16.2	176.2±14.9	180.0±0.0	162.2±46.9
	Latency trial 2 (sec)	180.0±0.0	173.8±24.1	179.6±1.7	174.2±23.3
Females					
Session 1 (learning phase)	Trials to criterion	3.0±0.0	3.1±0.3	3.1±0.4	3.3±0.7
	Latency trial 1 (sec)	35.3±29.3	62.6±55.8	41.8±42.8	35.5±29.2
	Latency trial 2 (sec)	180.0±0.0	170.2±39.0	170.3±35.1	179.8±0.6
	Failed to meet criterion	0	0	0	0
	Failed to cross	0	0	1	0
Session 2 (retention phase)	Trials to criterion	2.3±0.7	2.4±0.9	2.2±0.4	2.2±0.5
	Latency trial 1 (sec)	180.0±0.0	169.1±29.1	164.5±42.1	177.2±11.2
	Latency trial 2 (sec)	173.3±23.5	178.5±5.8	180.0±0.0	174.8±20.7

a Data (n=15-16) were obtained from Table 24 on pages 221-223 in the study report.

2. **Adult offspring – water maze:** No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the water maze test (Table 16b). The time to escape (duration) for the first trial of acquisition was increased ($p \leq 0.05$) for 150 ppm males, compared to controls, and the average number of trials to criterion (acquisition / learning phase) was non-statistically higher for this group (9.1 trials), compared to controls (7.4 trials). This difference from control is not considered a treatment-related effect since it only occurred at the low dose and only in males. Moreover, differences from control for the first trial would

not reflect an effect on either acquisition or retention, since the animals were completely naïve to the test during that trial.

Acquisition and retention were evident in control males and females. On the first test occasion, acquisition was evident in controls as a progressive decrease in the average time to escape (to reach the exit ramp) over successive trials. For males the average trial duration (time to escape) did not decrease from the first trial (an average 13.1 sec) to the second trial (an average 13.8 sec), however, trial duration decreased for subsequent trials (e.g., an average 6.7 sec for trial 5). For females, the average trial duration decreased from the first trial (an average 15.3 sec) to the second trial (an average 12.0 sec), with further reductions for subsequent trials (e.g., an average 6.2 sec for trial 5). As per standard procedure, animals that failed to demonstrate acquisition were not tested for retention. There were four males (one control and three low-dose) and no females that failed to demonstrate acquisition. On the second test occasion, retention was evident in control males as a reduction in the number of trials-to-criterion (6.0 vs. 7.4) and a shorter trial duration (10.5 vs. 13.1 sec) for the first trial, compared to the first trial of acquisition. For females, retention was evident by a decreased number of trials to criterion (6.2 vs. 7.7) and a shorter trial duration (7.6 vs. 15.3 sec) for the first trial, compared to the first trial of acquisition. These comparisons within the control group to verify changes in performance with experience were not subjected to statistical analysis.

TABLE 16b. Water maze performance(s) at PND 24 and 27 in offspring (mean±S.D.) [a]

Session/Parameter		Dose (ppm)			
		Control	150	1000	7000/4000
Males					
Learning Phase, PND 24	Trials to criterion	7.4±3.3	9.1±3.5	7.3±1.9	7.6±2.0
	Trial 1 – Errors	0.4±0.6	1.0±0.9	0.5±0.7	0.8±0.9
	Trial 1 – Duration (sec)	13.1±8.2	23.1±15.8* (↑76)	13.6±6.0	18.1±11.3
	Trial 2 – Errors	0.8±1.5	0.8±0.8	0.7±0.6	0.7±0.9
	Trial 2 – Duration (sec)	13.8±11.3	19.3±14.8	14.9±7.8	11.7±6.5
	Failed to meet criterion	1	3	0	0
Memory Phase, PND 27	Trials to criterion	6.0±1.7	5.6±1.3	5.8±1.1	5.8±1.4
	Trial 1 – Errors	0.5±0.7	0.1±0.3	0.6±0.9	0.1±0.3
	Trial 1 – Duration (sec)	10.5±6.8	7.8±5.4	11.4±9.1	6.9±4.9
	Trial 2 – Errors	0.2±0.6	0.4±0.8	0.1±0.3	0.4±1.0
	Trial 2 – Duration (sec)	4.7±3.1	5.5±4.1	5.6±4.3	5.7±6.0
Females					
Learning Phase, PND 24	Trials to criterion	7.7±2.4	7.6±2.3	7.3±2.1	6.9±2.0
	Trial 1 – Errors	0.9±0.7	0.7±0.9	0.7±0.7	0.7±0.9
	Trial 1 – Duration (sec)	15.3±8.2	18.7±13.1	18.2±9.4	17.5±11.6
	Trial 2 – Errors	0.6±0.7	0.7±0.9	0.6±0.9	0.5±0.6
	Trial 2 – Duration (sec)	12.0±8.3	15.3±13.7	13.5±8.9	12.2±9.9
	Failed to meet criterion	0	0	0	0
Memory Phase, PND 27	Trials to criterion	6.2±2.5	6.4±2.5	6.8±3.1	5.5±0.9
	Trial 1 – Errors	0.3±0.7	0.6±0.8	0.3±0.5	0.2±0.6
	Trial 1 – Duration (sec)	7.6±5.5	9.4±6.6	9.0±5.7	7.7±6.6
	Trial 2 – Errors	0.0±0.0	0.2±0.5	0.1±0.3	0.1±0.5
	Trial 2 – Duration (sec)	3.6±1.0	5.1±3.2	5.3±2.7	4.8±4.1

a Data (n=13-16) were obtained from Table 25 on pages 224-226 in the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.
* Statistically different from control, $p \leq 0.05$

5. <u>Ophthalmology</u>: No treatment-related ocular lesions were noted in males or females. Increased slight to minimal retinal degeneration was observed in 7000/4000 ppm females (2/9 affected) compared to 0 in the other female groups. However, in males 1/13 control also had retinal degeneration. No other dose-dependent increase in ocular lesions was observed. The Sponsor stated that all ophthalmologic findings were considered to be incidental and unrelated to treatment, due to lack of dose response, consistency by gender and/or because the incidence was within the range of historical control (data not provided). Retinal degeneration was not observed in the rat subchronic neurotoxicity study, which tested at a higher dose level (about 580 mg/kg/day).

6. Postmortem results

a. Brain weights: Decreased ($p \leq 0.05$) mean body weight was noted in the perfused 70000/4000 ppm males at PND 21 (↓11%; Table 17). In 21-day male pups, a 5% decrease in perfused brain weight was not considered adverse since it was seen only at the limit dose, was not statistically significant and was not sustained as there was an increase in brain weight in the 75-day old (termination) pups. In females, decreases observed in perfused brain weights were marginal (3.2% and 4% in the 21 and 75-day pups, respectively). At the 75-day termination, minor decreases of 4% were also noted in the non-perfused ($p \leq 0.05$) absolute brain weights in 7000/4000 ppm females; however, brain-to-body weight ratios were similar to control. These decreases in females were not considered adverse because they were marginal and were observed only at a high dose level. All other values in the treated groups were similar to controls.

TABLE 17. Mean (±SD) brain weight data [a]

Parameter	Dose (ppm)			
	Control	150	1000	7000/4000
Males				
Day 21 (perfused)				
Terminal body weight (g)	52.2±3.1	49.6±4.9	49.7±3.6	46.2±4.2* (↓11)
Brain weight (g)	1.457±0.068	1.393±0.048	1.432±0.059	1.386±0.088 (↓5)
Brain-to-body weight ratio	2.797±0.188	2.829±0.220	2.900±0.260	3.018±0.310
PND 75 (±5) (termination – perfused)				
Terminal body weight (g)	351.1±31.5	353.4±21.7	362.6±21.6	346.4±13.0
Brain weight (g)	1.798±0.122	1.804±0.077	1.884±0.096	1.854±0.123
Brain-to-body weight ratio	0.516±0.056	0.512±0.041	0.522±0.046	0.536±0.037
PND 75 (±5) (termination – non-perfused)				
Terminal body weight (g)	355.8±28.7	359.9±25.5	347.9±18.9	350.7±22.0
Brain weight (g)	1.957±0.101	1.956±0.081	2.008±0.081	1.950±0.144
Brain-to-body weight ratio	0.552±0.035	0.546±0.043	0.579±0.039	0.557±0.044
Females				
Day 21 (perfused)				
Terminal body weight (g)	51.3±5.2	51.8±3.7	48.9±4.4	48.4±2.1
Brain weight (g)	1.416±0.068	1.393±0.055	1.377±0.086	1.370±0.046 (↓3.2)
Brain-to-body weight ratio	2.776±0.200	2.698±0.165	2.829±0.204	2.836±0.130
PND 75 (±5) (termination – perfused)				
Terminal body weight (g)	211.2±15.8	210.3±14.0	207.4±11.3	202.9±12.4
Brain weight (g)	1.723±0.085	1.701±0.078	1.713±0.046	1.655±0.085 (↓4)
Brain-to-body weight ratio	0.818±0.045	0.811±0.049	0.828±0.050	0.818±0.051
PND 75 (±5) (termination – non-perfused)				
Terminal body weight (g)	213.5±11.4	214.1±16.3	214.7±13.6	207.9±17.7
Brain weight (g)	1.844±0.046	1.844±0.051	1.835±0.061	1.766±0.058* (↓4)
Brain-to-body weight ratio	0.865±0.043	0.865±0.051	0.858±0.052	0.854±0.064

a Data (n=9-10) were obtained from Tables OW1K, OW2K and OW3K-SUM on pages 939-944 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.

* Statistically different from control, $p \leq 0.05$

b. Neuropathology

1. **Macroscopic examination:** No treatment-related gross pathological findings were noted.

2. **Microscopic examination:** No treatment-related brain or non-brain micropathological findings were noted. At 7000/4000 ppm, there were several cases of minimal degeneration in the nervous system and one case of slight degeneration in the male right sciatic nerve. For most lesions, an equal or greater incidence of each lesion was noted in the controls, and only 1 additional finding was noted in the treated group when this was not the case. These findings (such as gasserion ganglion degeneration, cervical spinal cord degeneration and left tibial nerve degeneration) are common findings when observed at minimal severity and are considered incidental.

3. **Morphometric evaluation:** No treatment-related effect was noted during morphometric analysis (Table 18a and 18b). For perfused termination 7000/4000 ppm females, cerebrum length was decreased ($p \leq 0.05$) by 2% relative to controls. The Sponsor stated that this minor decrease was well within the historical control range for this laboratory (data not reported).

TABLE 18a. Mean (±SD) gross morphometric data [a]

Parameter	Dose (ppm)			
	Control	150	1000	7000/4000
Males				
PND 21				
Ant/post cerebrum length (mm)	13.76±0.28	13.74±0.16	13.64±0.33	13.56±0.31
Ant/post cerebellum (mm)	7.33±0.49	7.24±0.40	7.15±0.48	7.02±0.32
PND 75 (±5) (Termination-Perfused)				
Ant/post cerebrum length (mm)	14.53±0.54	14.66±0.43	14.99±0.39	14.81±0.45
Ant/post cerebellum (mm)	7.95±0.45	7.70±0.49	8.11±0.51	8.01±0.43
Females				
PND 21				
Ant/post cerebrum length (mm)	13.64±0.32	13.68±0.30	13.61±0.37	13.47±0.18
Ant/post cerebellum (mm)	7.28±0.32	6.93±0.37	7.18±0.42	7.16±0.28
PND 75 (±5) (Termination-Perfused)				
Ant/post cerebrum length (mm)	14.50±0.25	14.31±0.26	14.50±0.38	14.14±0.25* (↓2)
Ant/post cerebellum (mm)	7.80±0.30	7.81±0.26	7.73±0.36	7.80±0.48

a Data (9-10) were obtained from Tables BM1-SUM and BM2-SUM on pages 945-948 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.

* Statistically different from control, $p \leq 0.05$

TABLE 18b. Mean (±SD) microscopic morphometric data in mm [a]		
Parameter	**Control**	**7000/4000 ppm**
Males		
PND 21		
Frontal cortex	1.824±0.009	1.775±0.006
Parietal cortex	1.932±0.009	1.886±0.003
Caudate putamen	2.939±0.019	2.966±0.016
Hippocampal gyrus	1.574±0.010	1.568±0.009
Cerebellum	4.727±0.084	4.502±0.055
PND 75 (±5) (Termination-Perfused)		
Frontal cortex	1.595±0.012	1.618±0.011
Parietal cortex	1.757±0.011	1.824±0.007
Caudate putamen	3.246±0.012	3.339±0.018
Hippocampal gyrus	1.399±0.012	1.516±0.038
Cerebellum	4.396±0.079	4.682±0.110
Females		
PND 21		
Frontal cortex	1.832±0.004	1.847±0.006
Parietal cortex	1.909±0.009	1.922±0.003
Caudate putamen	2.949±0.010	3.014±0.039
Hippocampal gyrus	1.626±0.034	1.583±0.005
Cerebellum	4.879±0.051	4.941±0.028
PND 75 (±5) (Termination-Perfused)		
Frontal cortex	1.706±0.015	1.698±0.008
Parietal cortex	1.844±0.003	1.805±0.009
Caudate putamen	3.510±0.015	3.540±0.005
Hippocampal gyrus	1.560±0.021	1.550±0.016
Cerebellum	4.653±0.175	4.968±0.117

a Data (9-10) were obtained from Tables OW1K and OW2K-SUM pages 939-942 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Maternal toxicity was only observed at 7000/4000 ppm. Findings at 7000 ppm consisted of clinical signs (tremor, dilated pupils and nasal stain, repetitive chewing movements), a marked decrease in weight gain (42% from initiation of exposure to GD 20), decreased body weight (decreased up to 13% on GD 20 and 14% on LD 0) and food consumption (decreased 15% on LD 0-7). Clinical signs and body weight differences (↓3.7% on LD 21) recovered after the dose was reduced on LD 4. Offspring toxicity was also only observed at 7000/4000 ppm. At 7000 ppm, body weight was decreased in both sexes (combined) on PND 0 (↓13%) and PND 4 (↓16%), with a 22% lower body weight gain from PND 0-4, compared to controls. Recovery was evident after the high dose was reduced on PND 4, with a difference in weight of 6.1%, relative to control, on PND 21. The difference in weight persisted after weaning in males only, with recovery to within 3% of control at study termination. It is unclear whether the statistical difference in motor activity for males on PND 21 (↓29%) represents a treatment-related effect, since it only occurred on one day and there was no difference in females at any dietary level.

B. REVIEWER'S COMMENTS

In the maternal animals: No treatment-related effects were observed on mortality.

Toxicity was only observed in the 7000/4000 ppm group.

Treatment-related clinical signs were evident in the 7000 ppm animals as coarse tremor in four females, beginning on GD 14, dilated pupils in seven females, beginning on GD 15, and nasal stain in three females, beginning on GD 6. Treatment-related clinical signs during lactation were limited to dilated pupils in four high-dose females, which were apparent during gestation and resolved in all animals by LD 4 (when the dose was reduced to 4000 ppm) and repetitive chewing movements in one animal. Although chewing movements were apparent in only one animal as a clinical observation, it was evident by FOB in three 7000 ppm animals, which further supports this finding being a treatment-related effect. In the FOB, treatment-related effects were first evident on GD 6, with dilated pupils in three females. On GD 20, the following observations were made (# affected/30): dilated pupils (6), dilated pupils that were unresponsive to penlight (2), tremor (1) and repetitive chewing movements (3). There were no treatment-related effects during lactation at any dietary level.

Body weights were decreased ($p \leq 0.01$) on GD 13 and 20 (↓7-13%), resulting in a decreased ($p \leq 0.01$) cumulative body weight gain (GD 0-20) of 42%. During GD 6-13, food consumption increased ($p \leq 0.01$) by 159%. Although decreased body weight gain and increased food consumption suggests decreased food efficiency, the Sponsor stated that excessive feed spillage at the high dose occurred during the first week of treatment (GD 6-13) and resulted in an unreliable measure of food consumption for that week. Food consumption was similar to controls during GD 13-20. Therefore, the effect on food consumption is unclear. Body weights were decreased ($p \leq 0.05$, except on LD 14) throughout lactation by 4-14%, but increased (statistical analyses not performed [NP]) cumulative body weight gain (LD 0-21) of 71% was noted (calculated by the reviewer). Food consumption was decreased (NP) by 15% during LD 0-7, but was similar to controls thereafter. Beginning on LD 7, all 150 and 1000 ppm females were given grated feeders due to excessive feed spillage during the first week of lactation. Body weight for high-dose females was reduced an average 14% and 11%, compared to controls, on LD 0 and 4, respectively. The Sponsor stated that based on substantively reduced body weight (with associated concerns for the offspring) the high-dose was reduced on LD 4. After LD 4, the difference in body weight for high-dose animals progressively decreased with time. There was no effect on food consumption after the reduction of the high-dose for the remainder of lactation.

The number of litters was decreased (NS) by 17%.

The maternal LOAEL is 7000/4000 ppm (equivalent to 432 mg/kg/day), based on clinical signs and decreased body weights and body weight gains. The maternal NOAEL is 1000 ppm (equivalent to 83.8 mg/kg/day).

In the offspring: No treatment-related effects were observed on litter size, viability, clinical signs, developmental landmarks, functional observational battery, auditory startle reflex, learning and memory testing, ophthalmology, nervous system morphometric evaluation, or gross or microscopic pathology

Decreases ($p \leq 0.05$) in offspring body weights were noted in both sexes up to PND 4, post-culling (↓14-17%) and decreases (NS, except as noted) up to PND 21 in the males (↓6-10%; $p \leq 0.05$ at PND 11 and 21) and females (↓5-9%). Offspring postweaning male body weights were decreased by 3-8% ($p \leq 0.05$; except at PND 70), while female body weights in the treated groups were similar to controls.

Decreased motor activity ($p \leq 0.05$) in the 7000/4000 ppm males on PND 21 (↓29%) was observed. Although there were no significant changes at other time points, or in females, this difference from control was considered treatment-related because it was statistically significant and the average results for the high-dose males (228) was below the range of historical control from four developmental neurotoxicity studies (261-341) conducted during the same time period (2005-2007), while the results for controls were well within this range. All other aspects of motor activity, including habituation, were not considered to be affected by treatment.

In males, minor decreases in perfused brain weights (5%) were observed in 21-day pups. However, since the decreases were observed only at the limit dose, were not statistically significant and were not sustained (e.g., brain weights of 75-day old pups were increased), they were not considered adverse. In females, marginal decreases of 3.2% and 4% were seen in the brains of perfused 21 and 75-day pups, respectively. Minor decreases (4%) in the non-perfused ($p \leq 0.05$) brain weights in the 75-day 7000/4000 ppm females were observed, while brain-to-body weight ratios were similar to control. The brain weight changes in females were also not considered adverse due to their small magnitude and occurrence only at the limit dose; most also were not statistically significant.

The offspring LOAEL is 7000/4000 ppm (equivalent to 432 mg/kg/day), based on decreased body weights and decreased motor activity in male pups on PND 21. The offspring NOAEL is 1000 ppm (equivalent to 83.8 mg/kg/day).

This study is classified **Acceptable/Nonguideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 review of the positive control data.

C. **STUDY DEFICIENCIES:** Historical ophthalmology and brain morphology data were not provided; however, this lack was considered a minor deficiency that does not affect the conclusions of this review.

ATTACHMENT

The following attachment contains pages 196-199 of MRID 47443311.

TECHNICAL GRADE BCS-AA10717
Summary Interval Motor Activity for Male Rats, Postnatal Day 13
Study Number 07-D72-KC

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	13 ± 14	20 ± 22	18 ± 19	7 ± 13	6 ± 9	5 ± 7
150 PPM	25 ± 25	18 ± 23	16 ± 25	9 ± 16	9 ± 16	9 ± 12
1000 PPM	19 ± 14	17 ± 20	14 ± 16	13 ± 24	17 ± 23	20 ± 29
7000/4000 PPM	26 ± 29	21 ± 30	23 ± 23	18 ± 21	18 ± 24	12 ± 19

* Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Postnatal Day 0 = 06/04/2007
Number of Rats/Group: 20/0 PPM 20/150 PPM 20/1000 PPM 19/7000/4000 PPM

TECHNICAL GRADE BCS-AA10717
Summary Interval Motor Activity for Male Rats, Postnatal Day 17
Study Number 07-D72-KC

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	84 ± 49	53 ± 31	37 ± 37	43 ± 38	37 ± 40	30 ± 31
150 PPM	68 ± 38	38 ± 36	24 ± 29	11 ± 21	15 ± 24	18 ± 29
1000 PPM	63 ± 37	41 ± 29	19 ± 17	16 ± 14	25 ± 25	24 ± 24
7000/4000 PPM	62 ± 38	29 ± 26	25 ± 30	15 ± 22	25 ± 29	28 ± 30

* Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Postnatal Day 0 = 06/04/2007
Number of Rats/Group: 20/0 PPM 20/150 PPM 20/1000 PPM 19/7000/4000 PPM

TECHNICAL GRADE BCS-AA10717
Summary Interval Motor Activity for Male Rats, Postnatal Day 21
Study Number 07-D72-KC

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	112 ± 20	58 ± 20	46 ± 29	51 ± 45	29 ± 24	26 ± 27
150 PPM	103 ± 31	53 ± 33	31 ± 28	26 ± 25	28 ± 27	32 ± 32
1000 PPM	101 ± 19	52 ± 31	37 ± 27	21 ± 19	20 ± 22	27 ± 25
7000/4000 PPM	94 ± 35	39 ± 27	31 ± 23	28 ± 23	12 ± 15	23 ± 31

* Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Postnatal Day 0 = 06/04/2007
Number of Rats/Group: 20/0 PPM 20/150 PPM 20/1000 PPM 19/7000/4000 PPM

TECHNICAL GRADE BCS-AA10717
Summary Interval Motor Activity for Male Rats, Postnatal Day 60
Study Number 07-D72-KC

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	113 ± 28	100 ± 46	103 ± 53	99 ± 46	81 ± 30	73 ± 32
150 PPM	115 ± 22	93 ± 31	105 ± 43	86 ± 32	88 ± 47	74 ± 26
1000 PPM	99 ± 17	92 ± 23	101 ± 40	97 ± 41	81 ± 36	73 ± 18
7000/4000 PPM	122 ± 23	106 ± 22	107 ± 28	96 ± 28	97 ± 27	87 ± 31

* Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Postnatal Day 0 = 06/04/2007
Number of Rats/Group: 20/0 PPM 20/150 PPM 20/1000 PPM 19/7000/4000 PPM